

Short Communications / Kort Mededelings**Effect of delayed sponge withdrawal on the superovulatory response to pregnant mare serum gonadotrophin and on embryo recovery in sheep**

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Forty-five ewes (29 Romanov × Dorper, 9 Karakul, and 7 Dorper) were divided into four groups, synchronized using progestagen sponges and superovulated with 1 000 IU pregnant mare serum gonadotrophin. Sponges were withdrawn on days 13, 14, 15 and 16 (in groups 1—4, respectively) after sponge insertion (day 0). Fixed-time cervical as well as laparoscopic AI was performed. The fallopian tubes and uterus were flushed eight days after sponge withdrawal. Total ovarian response (follicles >8 mm and corpora lutea), total corpora lutea, total embryo and ova recovery, total transferable embryos and left and right ovarian reactions (follicles >8 mm and corpora lutea; corpora lutea; embryo and ova recovery; transferable embryos for each ovary) were noted per ewe in each group. No significant differences were observed between the four groups in all parameters measured. When data were pooled, the total ovarian response was 416, number of corpora lutea was 274, the number of ova and embryos recovered was 141 (recovery rate 51.5%) and the number of transferable embryos was 102. No significant differences between left and right ovarian reactions were detected within and between groups.

Vyf-en-veertig ooie (29 Romanov × Dorper, 9 Karakoel en 7 Dorper) is in vier groepe verdeel, gesinchroniseer met progeste-roonsponse en gesuperovuleer met 1 000 IE dragtige merrieserum-gonadotrofien. Sponse is onttrek op dae 13, 14, 15 en 16 (in groepe 1—4, respektiewelik) na sponsing (dag 0). Die ooie is op vasgestelde tye servikaal en laparoskopies geïnsimineer. Die fallopiese buise en baarmoeder is agt dae na sponsonttrekking gespoel. Die totale ovariumrespons (follikels >8 mm en corpora lutea), totale corpora lutea, totale embryo- en ova-herwinning, totale oorplaasbare embrio's en linker- en regterovariumreaksies (follikels >8 mm en corpora lutea; corpora lutea; embrio- en ova-herwinning; oorplaasbare embrio's vir elke ovarium) is bepaal per ooi vir elke groep. Geen betekenisvolle verskille tussen die vier groepe is opgemerk vir die parameters wat gemeet is nie. Wanneer waardes gepool is, was die totale ovariumrespons, 416; die aantal corpora lutea, 274; die aantal ova en embrio's herwin, 141 (herwinningspersentasie 51.5%); en die aantal oorplaasbare embrio's, 102. Geen betekenisvolle verskille tussen linker- en regterovariumreaksies binne en tussen groepe is opgemerk nie.

Keywords: Embryo, PMSG, sheep, superovulation.

The techniques of embryo flushing, transfer and freezing depend on successful superovulation and embryo recovery. When large numbers of embryos are required on a daily basis for biotechnological applications such as embryo freezing and cloning, and consequently numerous sheep need to be superovulated, progestagen sponge insertion in all ewes on the same day would ease the management of the donor ewes. In this study the effect of length of sponge insertion on the superovulatory response in ewes was examined using pregnant mare serum gonadotrophin (PMSG) in the breeding season and embryo production was evaluated.

Forty-five ewes (29 Romanov × Dorper, 9 Karakul and 7 Dorper) were divided into four groups. Allocation of ewes to treatment groups was according to breed and age. The ewes were all in a similar condition. The ewes were synchronized using progestagen intravaginal sponges (medroxyprogesterone acetate). Twenty-four hours prior to sponge withdrawal, each ewe received 1 000 IU PMSG intramuscular. Sponges were withdrawn on days 13, 14, 15 and 16 (in groups 1—4, respectively), where day 0 was day of sponge insertion. Sheep losing sponges were discarded from the study. Ewes were teased twice, at 12 h and 24 h, after sponge withdrawal. Fixed-time artificial insemination (AI) was performed at 36 h (cervical AI 120—260 million live sperm / dose) and at 48 h (laparoscopic AI 24—90 million live sperm / horn) after sponge withdrawal. Semen was collected on a daily basis from fertile rams and an egg yolk-citrate diluent was used (Evans & Maxwell, 1987). After AI, the ewes were placed with fertile rams until slaughter eight days after sponge withdrawal. The reproductive tracts were removed and the fallopian tubes and uteri were flushed with 200 ml Dulbecco's phosphate buffered saline as modified by Tervit *et al.* (1972). Total ovarian response (follicles >8 mm and corpora lutea), total ovulations (corpora lutea), total ova and embryo recovery, total transferable embryos (graded fair or better), and left and right ovarian reactions (follicles >8 mm and corpora lutea; corpora lutea; embryos and ova recovered; transferable embryos for each ovary) were noted per ewe in each group. The embryos were classified according to stage of development.

The oestrous response to the synchronization regime was 79% (Table 1). Only two sheep lost sponges (4.4%). Although a mucoid discharge was noted on sponge withdrawal, this was not foul-smelling and should not affect embryo quality (Scudamore, 1988). No significant differences between groups were detected with respect to oestrous response after sponge withdrawal.

Table 1 Effect of duration of sponge insertion on oestrous response (figures in brackets denote percentages)

Group	Sponge days	Oestrous response		Total ewes in each group
		12 h	24 h	
1	13	0 (0)	8 (89)	9
2	14	2 (17)	10 (83)	12
3	15	0 (0)	8 (73)	11
4	16	1 (9)	8 (73)	11
Total		3 (7)	34 (79)	43

Table 2 Superovulatory response of ewes treated with 1 000 IU PMSG (n denotes the number of ewes within each group)

Group	Sponge days	Total ovarian response ^a		Total corpora lutea		Total embryos and ova recovered		Total transferable embryos	
		Mean \pm SD (n)	Range	Mean \pm SD (n)	Range	Mean \pm SD (n)	Range	Mean \pm SD (n)	Range
1	13	10.2 \pm 6.3 (9)	2—22	6.7 \pm 4.2 (9)	1—12	2.8 \pm 2.1 (9)	0—7	2.3 \pm 2.2 (9)	0—7
2	14	10.8 \pm 5.7 (12)	5—20	7.2 \pm 4.4 (12)	2—16	3.4 \pm 3.0 (12)	0—9	2.7 \pm 2.4 (10)	0—6
3	15	10.3 \pm 8.0 (11)	3—30	7.0 \pm 6.8 (11)	0—22	4.2 \pm 4.8 (10)	1—17	2.6 \pm 4.8 (10)	0—16
4	16	7.5 \pm 5.5 (11)	0—20	5.1 \pm 4.1 (10)	2—13	3.3 \pm 3.8 (10)	0—10	3.4 \pm 3.3 (8)	1—9
Mean		9.7 \pm 6.3 (43)	0—30	6.5 \pm 4.9 (42)	0—22	3.4 \pm 3.4 (41)	0—17	2.7 \pm 3.2 (37)	0—16
Total		416		274		141		102	

^a Follicles > 8 mm and corpora lutea per ewe.

Table 3 Classification of embryos recovered from ewes following varying periods of exposure to progestagen (figures in brackets denote percentages; day 0 is day of oestrus)

Day	<32 Cells	Morula	Early blastocyst	Blastocyst	Expanded blastocyst	Unfertilized ova	Degenerating embryos	Total
<6	3 (16.7)	8 (44.4)	2 (11.1)	1 (5.6)	1 (5.5)	1 (5.6)	2 (11.1)	18
6	10 (8.8)	65 (57.0)	10 (8.8)	7 (6.1)	0 (0.0)	9 (7.9)	13 (11.4)	114
6½	0 (0.0)	7 (77.8)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	9
Total	13 (9.2)	80 (56.7)	13 (9.2)	8 (5.7)	1 (0.7)	10 (7.1)	16 (11.4)	141

The various parameters measured for each group are shown in Table 2. As can be seen from Table 2, the variation in degree of total ovarian response (range 0—30) and in the number of corpora lutea (range 0—22) was very high and parallels the variation described by Maxwell *et al.* (1990). No significant differences between groups were detected with respect to total ovarian response, total corpora lutea, total number of ova and embryos recovered, and total number of transferable embryos. The reactions of the left and right ovaries did not differ within or between groups.

Only one sheep did not respond to superovulation (no visible follicles and corpora lutea on both ovaries). By definition, superovulation means three or more ovulations per donor (Betteridge, 1980) and this was achieved in 33 (76.7%) of the ewes. The data in Table 3 show the stage of development of the embryos which were recovered from all groups. Most embryos recovered were in the morula stage. This signifies that for more advanced embryos such as blastocysts and expanded blastocysts, flushing must occur later than day 6½ post-oestrus when using 1 000 IU PMSG. Later stage embryos (blastocysts and expanded blastocysts) have a better survival rate after freezing (Li *et al.*, 1990) and result in more pregnancies when transferred unfrozen to recipients (Armstrong & Evans, 1983).

When data from all four groups were pooled, the total ovarian response was 416, but only 274 (66%) follicles ovulated. The recovery rate of ova and embryos (141 in total) was only 51.5% of corpora lutea counted. Factors that could have played a role in this low recovery rate include intra-uterine insemination (Walker *et al.*, 1989) and the long half-life of PMSG (Quirke & Hanrahan, 1975; Armstrong *et al.*, 1983a; 1983b; Maxwell *et al.*, 1990). These results indicate that length of sponge insertion (13—16 days) does not have a significant effect on the superovulatory response and embryo production when 1 000 IU PMSG is used.

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